

**Manuscript version: Author's Accepted Manuscript**

The version presented in WRAP is the author's accepted manuscript and may differ from the published version or Version of Record.

**Persistent WRAP URL:**

<http://wrap.warwick.ac.uk/110005>

**How to cite:**

Please refer to published version for the most recent bibliographic citation information. If a published version is known of, the repository item page linked to above, will contain details on accessing it.

**Copyright and reuse:**

The Warwick Research Archive Portal (WRAP) makes this work by researchers of the University of Warwick available open access under the following conditions.

Copyright © and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable the material made available in WRAP has been checked for eligibility before being made available.

Copies of full items can be used for personal research or study, educational, or not-for-profit purposes without prior permission or charge. Provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.

**Publisher's statement:**

Please refer to the repository item page, publisher's statement section, for further information.

For more information, please contact the WRAP Team at: [wrap@warwick.ac.uk](mailto:wrap@warwick.ac.uk).

# Input-Dependent Structural Identifiability of Nonlinear Systems

Alejandro F. Villaverde<sup>1</sup>, Neil D. Evans<sup>2</sup>, Michael J. Chappell<sup>2</sup>, and Julio R. Banga<sup>1</sup>

**Abstract**—A dynamic model is structurally identifiable if it is possible to infer its unknown parameters by observing its output. Structural identifiability depends on the system dynamics, output, and input, as well as on the specific values of initial conditions and parameters. Here we present a symbolic method that characterizes the input that a model requires to be structurally identifiable. It determines which derivatives must be non-zero in order to have a sufficiently exciting input. Our approach considers structural identifiability as a generalization of nonlinear observability and incorporates extended Lie derivatives. The methodology assesses structural identifiability for time-varying inputs and, additionally, it can be used to determine the input profile that is required to make the parameters structurally locally identifiable. Furthermore, it is sometimes possible to replace an experiment with time-varying input with multiple experiments with constant inputs. We implement the resulting method as a MATLAB toolbox named STRIKE-GOLDD2. This tool can assist in the design of new experiments for the purpose of parameter estimation.

## I. INTRODUCTION

The concept of *structural identifiability* describes whether it is theoretically possible to determine the true value of a parameter from observations of the model output [6], [30]. Structural identifiability is a mathematical property determined by the model equations, which define the mapping from parameters to outputs; unlike *practical identifiability*, it is not affected by the quantity or quality of the data. Structurally unidentifiable parameters are a possible source of error in predictions made by dynamic models, and compromise their ability to provide biological insight [25].

Since [3], many methods have been proposed to analyse the structural identifiability of biological models. Some of them, such as the similarity transformation approach [9], [32] and direct test [7], are applicable to autonomous systems, i.e. systems with no input. Other approaches, such as those based on power series [17], differential algebra [4], [15], implicit functions [31], or differential geometry [27], can be applied to systems with external inputs. Additionally, it is possible to assess identifiability for a fully defined experiment (i.e. with specific values for initial conditions and inputs) with numerical approaches based on profile likelihoods [18] or the sensitivity matrix [23]. There are a number of software tools

implementing some of the aforementioned methodologies. Examples designed for determining global identifiability include DAISY [4], GenSSI [5], and COMBOS [16]; for testing for local identifiability, EAR [13], STRIKE-GOLDD [27], or Data2Dynamics [19].

These methods analyse models with a given input-output configuration. The term *qualitative* experiment design was coined to refer to the selection of input and output ports in order to maximize the number of *structurally* identifiable parameters [22], [29]. In fact, to fully define the qualitative aspects of an experiment design that guarantees structural identifiability we must determine:

- (a) which type of perturbations must be applied to the system (input definition),
- (b) which states, or combinations thereof, must be measured (output definition), and
- (c) which initial conditions of the states are appropriate.

We note that, once structural identifiability has been guaranteed by taking into account the aforementioned issues (a, b, c), a full experiment definition still requires deciding on other aspects, such as the number and timing of the samples, detailed characterization of the inputs, and so on. This task is known as *quantitative* experiment design, and can be used for maximizing *practical* identifiability.

In the present paper we address a different, although related, problem. We seek to determine, *a priori*, what mathematical form the inputs must have in order to guarantee *structural* identifiability, that is, the question (a) in the above list. In regard to the other points, question (b) has been studied in [1], [2], and question (c) in [8], [21], [26]. As an example of the problem we want to address, imagine a model for which a constant input is not sufficiently exciting, and requires a ramp input to make its output different for different parameters. To a certain extent, this situation resembles the relationship between structural identifiability and initial conditions (item ‘c’). Generally, methods that analyse structural identifiability yield results that are valid for *almost all* initial conditions; however, a model classified as structurally identifiable may lose structural identifiability when started from particular initial conditions [8], [21], [26]. Likewise, for the case of inputs, structural identifiability analysis methods can determine whether a model is structurally identifiable *provided that sufficiently exciting inputs are applied* – but it is not straightforward to characterize the necessary inputs with the existing implementations of these methods [4], [15]. Our aim is to determine this qualitative information analytically, without performing a numerical, or quantitative, optimal experimental design (OED) procedure. In this way

\*This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 686282 (“CANPATHPRO”)

<sup>1</sup>Alejandro F. Villaverde and Julio R. Banga are with Bioprocess Engineering Group, IIM-CSIC, Vigo 36208, Spain afvillaverde@iim.csic.es, julio@iim.csic.es.

<sup>2</sup>Neil D. Evans and Michael J. Chappell are with School of Engineering, University of Warwick, Coventry CV4 7AL, UK neil.evans@warwick.ac.uk, m.j.chappell@warwick.ac.uk.

we will be able to rule out insufficiently exciting inputs *a priori*, removing them from consideration in a subsequent quantitative OED.

We address this problem by building on an existing tool, STRIKE-GOLDD [27], which is a MATLAB toolbox that implements a structural identifiability analysis methodology based on differential geometry. It considers structural identifiability as an extension of observability, and assesses this by testing whether an observability-identifiability matrix has full rank. Here we show that, in its original form, STRIKE-GOLDD may wrongly classify an identifiable model as unidentifiable if a time-varying input is required for identification, which happens when a constant input does not excite the system dynamics sufficiently. This limitation originates from the definitions of the Lie derivatives used to build the observability-identifiability matrix, which are not appropriate for time-varying inputs. We modify the method by using extended Lie derivatives, which correctly analyse the effect of time-varying inputs. Importantly, in this way it is possible to obtain information about the type of input that is necessary in order to guarantee structural identifiability, by studying the dependence of the rank of the identifiability matrix on the expression for the (potentially time-varying) inputs. We demonstrate this capability with several models which are shown to require time-varying inputs in order to be structurally identifiable. We also show that it is sometimes possible to avoid the requirement of time-varying inputs by approximating it with multiple experiments with constant inputs of different values. This is particularly useful for biological systems, since the ability to change inputs over time is typically limited in this context.

The method proposed here was partly described in a short paper presented at the FOSBE 2018 conference [28]. With respect to that paper, the present manuscript contains the following extensions: (i) it extends the methodology to multidimensional inputs, enabling it to specify different time dependencies for every individual input; (ii) it shows that the requirement of time-varying inputs may sometimes be replaced with multiple experiments with constant inputs; (iii) it provides an implementation of the method as a software toolbox, STRIKE-GOLDD2; and (iv) it demonstrates the application of the resulting tool to nonlinear, nonrational models.

## II. METHODOLOGY

### A. Notation

Dynamic biological processes may be described by ordinary differential equations (ODEs) as follows:

$$\text{model } M : \begin{cases} \dot{x}(t) &= f(x(t), u(t), p), \\ y(t) &= g(x(t), p), \\ x_0 &= x(t_0, p) \end{cases} \quad (1)$$

where  $f$  and  $g$  are analytic vector functions,  $p \in \mathbb{R}^q$  is the parameter vector,  $u(t) \in \mathbb{R}^r$  the input vector,  $x(t) \in \mathbb{R}^n$  the state variable vector, and  $y(t) \in \mathbb{R}^m$  the output vector. We drop the dependence on  $p$  for ease of notation.

### B. Background on observability

Conceptually, a model is observable if it is possible to determine its internal state vector  $x$  by observing its output vector  $y$ . Observability can be evaluated by calculating the rank of an observability matrix  $O(x)$  that represents a map between the model output  $y$  (and its derivatives  $\dot{y}, \ddot{y}, \dots$ ) on the one hand, and its state  $x$  on the other. If  $O(x)$  has full rank, then the model is observable. The observability matrix contains the partial derivatives of the output (and its derivatives) with respect to the states:

$$\mathcal{O}(x(t)) = \begin{pmatrix} \frac{\partial}{\partial x} y(t) \\ \frac{\partial}{\partial x} \dot{y}(t) \\ \frac{\partial}{\partial x} \ddot{y}(t) \\ \vdots \\ \frac{\partial}{\partial x} y^{(n-1)}(t) \end{pmatrix} \quad (2)$$

**Theorem 1.** *The Observability Rank Condition (ORC): If the system  $M$  given by (1) satisfies  $\text{rank}(\mathcal{O}(x_0)) = n$ , where  $\mathcal{O}$  is defined by (2), then it is (locally) observable around  $x_0$  [24].*

### C. Structural identifiability: definitions

**Definition 1.** A parameter  $p_i$  is structurally globally identifiable (s.g.i.) if it can be uniquely determined from the system output, that is, if for almost any  $p^* \in \mathbb{R}^q$  (i.e., for any  $p$  except those belonging to a set of measure zero) the following property holds for all  $t$  and all admissible inputs  $u$  [15]:

$$y(t, \hat{p}) = y(t, p^*) \Rightarrow \hat{p}_i = p_i^* \quad (3)$$

**Definition 2.** A parameter  $p_i$  is structurally locally identifiable (s.l.i.) if for almost any  $p^*$  there is a neighbourhood  $V(p^*)$  in which (3) holds.

**Definition 3.** A parameter  $p_i$  is structurally unidentifiable (s.u.) if (3) does not hold in any neighbourhood of  $p^*$ .

**Definition 4.** A model  $M$  is s.g.i. if all its parameters are s.g.i.; it is s.u. if at least one of its parameters is s.u.; and it is s.l.i. if all its parameters are either s.l.i. or s.g.i. and at least one of them is not s.g.i..

**Remark 1.** *Structural local identifiability and observability are generic properties that hold for all points in parameter or state space (except possibly for a subset of measure zero). As an example, consider the model given by  $\dot{x} = u$ ,  $y = [\cos(x), \sin(x)]^T$ . This model is locally observable: every  $x_A$  can be distinguished from nearby states. However it is not globally observable:  $x_A$  cannot be distinguished from  $x_B = x_A + 2k\pi$  for any integer  $k$  [11].*

### D. Structural identifiability as generalized observability

The model's structural identifiability can be evaluated in the same way as its observability. To this end, we consider the parameters  $p_i$  as additional states with zero dynamics,  $\dot{p}_i = 0$ , i.e., we augment the state variable vector as  $\tilde{x} = (x, p)$ . The augmented (or generalized) observability-identifiability matrix,  $\mathcal{O}_I(\tilde{x})$ , is then defined for constant inputs as:

$$\mathcal{O}_I(\tilde{x}) = \begin{pmatrix} \frac{\partial}{\partial \tilde{x}} y(t) \\ \frac{\partial}{\partial \tilde{x}} \dot{y}(t) \\ \frac{\partial}{\partial \tilde{x}} \ddot{y}(t) \\ \vdots \\ \frac{\partial}{\partial \tilde{x}} y^{(n+q-1)}(t) \end{pmatrix} \quad (4)$$

**Theorem 2.** *Observability-Identifiability Condition (OIC):* if the system  $M$  given by (1) with constant input  $u$  satisfies  $\text{rank}(\mathcal{O}_I(\tilde{x}_0)) = n + q$ , with  $\mathcal{O}_I(\tilde{x}_0)$  given by (4), then it is observable and (at least locally) identifiable in a neighbourhood  $N(\tilde{x}_0)$  of  $\tilde{x}_0$ .

**Remark 2.** The OIC can be used to determine the structural identifiability of the model as a whole and of its parameters individually. Each column in (4) contains the partial derivatives with respect to a particular parameter (or state). Therefore, if deleting a given column does not change the rank of  $\mathcal{O}_I$ , it means that the corresponding parameter is unidentifiable.

#### E. Calculating $\mathcal{O}_I$ with extended Lie derivatives

For nonlinear systems  $\mathcal{O}_I$  can be calculated using differential geometry tools.

*Definition 5.* The Lie derivative of  $g$  with respect to  $f$  is defined by:

$$L_f g(\tilde{x}) = \frac{\partial g(\tilde{x})}{\partial \tilde{x}} f(\tilde{x}, u). \quad (5)$$

Higher order Lie derivatives can be recursively calculated as:

$$\begin{aligned} L_f^2 g(\tilde{x}) &= \frac{\partial L_f g(\tilde{x})}{\partial \tilde{x}} f(\tilde{x}, u), \\ &\vdots \\ L_f^i g(\tilde{x}) &= \frac{\partial L_f^{i-1} g(\tilde{x})}{\partial \tilde{x}} f(\tilde{x}, u). \end{aligned} \quad (6)$$

Note that if the input vector contains only *constant* inputs,  $u(t) = u$ , the expressions for the Lie derivatives used in (5, 6) correspond to the output derivatives, i.e.  $y^{(i)}(t) = L_f^i g(\tilde{x})$ . Structural identifiability analysis methods such as STRIKE-GOLDD [27] have used this equality to calculate  $\mathcal{O}_I$ . However, for time-varying inputs the equality between output derivatives and the Lie derivatives defined in (5,6) does not hold. To preserve this correspondence for time-varying inputs  $u(t)$  we modify the definition of the Lie derivative as follows:

*Definition 6.* The extended Lie derivative [13] is defined by:

$$L_f g(\tilde{x}) = \frac{\partial g(\tilde{x})}{\partial \tilde{x}} f(\tilde{x}, u) + \sum_{j=0}^{j=\infty} \frac{\partial g(\tilde{x})}{\partial u^{(j)}} u^{(j+1)}. \quad (7)$$

In the second term of the sum within (7),  $u^{(j)}$  and  $u^{(j+1)}$  denote the  $j^{\text{th}}$  and  $(j+1)^{\text{th}}$  derivatives of the input, respectively (note that we write  $u$  instead of  $u(t)$  to ease the notation). Higher order extended Lie derivatives are calculated using:

$$L_f^i g(\tilde{x}) = \frac{\partial L_f^{i-1} g(\tilde{x})}{\partial \tilde{x}} f(\tilde{x}, u) + \sum_{j=0}^{j=\infty} \frac{\partial L_f^{i-1} g(\tilde{x})}{\partial u^{(j)}} u^{(j+1)} \quad (8)$$

**Remark 3.** Since the output function  $g$  does not depend on the input directly (i.e. it is  $g(\tilde{x})$ , not  $g(\tilde{x}, u)$ ), it holds that

$$\sum_{j=0}^{j=\infty} \frac{\partial g(\tilde{x})}{\partial u^{(j)}} u^{(j+1)} = 0 \quad (9)$$

and the first order extended Lie derivative defined in (7) is identical to its non-extended counterpart (5). However, the summation term is not necessarily zero for higher order extended Lie derivatives (8),  $L_f^i g(\tilde{x})$ , if  $i \geq 2$ .

**Remark 4.** It can be seen that the  $i^{\text{th}}$  Lie derivative,  $L_f^i g(\tilde{x})$ , may contain input derivatives only up to order  $(i-2)$ . Hence, the infinite summation in (8) can be truncated in practice at  $j = i - 2$ , and  $L_f^i g(\tilde{x})$  is calculated as:

$$L_f^i g(\tilde{x}) = \frac{\partial L_f^{i-1} g(\tilde{x})}{\partial \tilde{x}} f(\tilde{x}, u) + \sum_{j=0}^{j=i-2} \frac{\partial L_f^{i-1} g(\tilde{x})}{\partial u^{(j)}} u^{(j+1)} \quad (10)$$

Extended Lie derivatives can be used to calculate  $\mathcal{O}_I(\tilde{x})$  for nonlinear models with inputs that are polynomial functions of time as follows:

$$\mathcal{O}_I(\tilde{x}) = \begin{pmatrix} \frac{\partial}{\partial \tilde{x}} y(t) \\ \frac{\partial}{\partial \tilde{x}} \dot{y}(t) \\ \frac{\partial}{\partial \tilde{x}} \ddot{y}(t) \\ \vdots \\ \frac{\partial}{\partial \tilde{x}} y^{(n+q-1)}(t) \end{pmatrix} = \begin{pmatrix} \frac{\partial}{\partial \tilde{x}} g(\tilde{x}) \\ \frac{\partial}{\partial \tilde{x}} (L_f g(\tilde{x})) \\ \frac{\partial}{\partial \tilde{x}} (L_f^2 g(\tilde{x})) \\ \vdots \\ \frac{\partial}{\partial \tilde{x}} (L_f^{n+q-1} g(\tilde{x})) \end{pmatrix} \quad (11)$$

#### F. Structural identifiability analysis for experiment design

The method presented in the preceding subsections can assist input design. Extended Lie derivatives (8) can be used to characterize the type of time dependency that is needed for the input to enable structural identifiability, by determining which of its derivatives must be non-zero. We can do this by setting to zero in  $\mathcal{O}_I$  (11) the derivatives of the input of order higher than a given one and recalculating  $\text{rank}(\mathcal{O}_I)$ . This condition ( $u^{(k)} = 0$ ) holds for any time instant, not just momentarily; as a result, higher order derivatives ( $u^{(k+1)}, u^{(k+2)}, \dots$ ) are also zero. For example, if  $\mathcal{O}_I$  has full rank for  $\dot{u} = 0$ , a constant input is sufficient for structural identifiability. If, however,  $\mathcal{O}_I$  has full rank for  $\{\dot{u} \neq 0, \ddot{u} = 0\}$ , but not for  $\dot{u} = 0$ , then a ramp input is necessary and sufficient, and a constant input is not. Note that this approach does not make *a priori* assumptions about the validity of the OIC for specific inputs. It directly assesses whether a particular form of  $u(t)$  leads to a decrease in the rank of  $\mathcal{O}_I$  with respect to the most generic case, which is assumed to be sufficiently exciting. The effect of a specific input can be tested by entering the corresponding expression for  $u(t)$  in (4).

**Remark 5.** Note that the input  $u(t)$  is in general a multi-dimensional vector, so these considerations apply to each of the inputs individually.

### G. Replacing time-varying inputs with constant inputs

An experiment with a time-varying input can be piecewise approximated by a series of experiments with constant input. In some cases both set-ups are indeed equivalent for the purpose of structural identifiability. A multi-experiment setting can be specified by modifying the model to include as many replicates of the state, output, and input vectors as experiments [14], which increases the dimension of the corresponding matrix  $\mathcal{O}_I$ . The first tool to implement multi-experiment structural identifiability analysis was GenSSI 2.0 [14].

### H. Implementation

The methodology presented in this section has been implemented in a MATLAB toolbox called STRIKE-GOLDD2, which is available at <https://sites.google.com/site/strikegolddtoolbox/>. The download includes the examples analysed in this paper and a complete documentation.

## III. RESULTS

Here we demonstrate the use of the STRIKE-GOLDD2 toolbox with several case studies: a 4-parameter linear model of a physiological system, a 10-parameter nonlinear model of ion channels, and an 8-parameter nonlinear model of a bioprocess with five inputs. The two latter models are nonlinear and non-rational in structure, which makes their symbolic analysis more challenging. Fig. 1 shows conceptual versions of the three models. To clarify specific aspects of the methodology we include as an Appendix a detailed analysis of the bioprocess model, which is provided as Supplementary Information due to page limitations.

### A. A two compartment model

This model represents a physiological system with two compartments (i.e. two states, of which one is measured) and one input. The model equations are given by:

$$M_1 : \begin{cases} \dot{x}_1 &= -(k_{1e} + k_{12}) \cdot x_1 + k_{21} \cdot x_2 + b \cdot u, \\ \dot{x}_2 &= k_{12} \cdot x_1 - k_{21} \cdot x_2, \\ y &= x_1 \end{cases} \quad (12)$$

where the unknown parameter vector is given by  $p = (k_{1e}, k_{12}, k_{21}, b)$ . Note that the initial condition of the unmeasured state,  $x_2(0)$ , is also considered unknown. The model diagram is shown in Fig. 1.A.

Calculating the matrix  $\mathcal{O}_I$  in Eq. (4) for  $M_1$  with 5 extended Lie derivatives, as in (7–8), yields a  $6 \times 6$  matrix with  $\text{rank}(\mathcal{O}_I) = 6$ . Therefore the observability-identifiability rank condition (OIC) is satisfied. This means that the model is structurally identifiable (as long as the input is sufficiently exciting). When the input is constant,  $\mathcal{O}_I$  can be calculated using (5–6); in this case  $\text{rank}(\mathcal{O}_I) = 5$  and the model is unidentifiable.

The practical meaning of these results is that, in order to determine the values of the parameters  $(k_{1e}, k_{12}, k_{21}, b)$  by measuring the model output ( $y = x_1$ ), it is necessary

to perform an experiment with time-varying input ( $\dot{u} \neq 0$ ). Moreover, we can characterize the type of time dependency that is needed for the input to enable structural identifiability. We can do this by replacing the higher order derivatives of the input in  $\mathcal{O}_I$  by zero and recalculating  $\text{rank}(\mathcal{O}_I)$ . For the model  $M_1$  this procedure yields  $\text{rank}(\mathcal{O}_I) = 6$  even if  $\ddot{u} = 0$ , as long as  $\dot{u} \neq 0$ ; however,  $\text{rank}(\mathcal{O}_I)$  reduces to 5 if  $\dot{u} = 0$ . Thus we know that a ramp input ( $u(t) = c_1 \cdot t + c_2$ , where  $c_1$  and  $c_2$  are arbitrary constants) suffices for structural identifiability of the parameters, but a constant input ( $u(t) = c_2$ ) does not. The two time course plots in Fig. 2 illustrate this fact. For this model we could not find a set of experiments with constant inputs that was equivalent to a time-varying input. The following example illustrates the opposite situation.

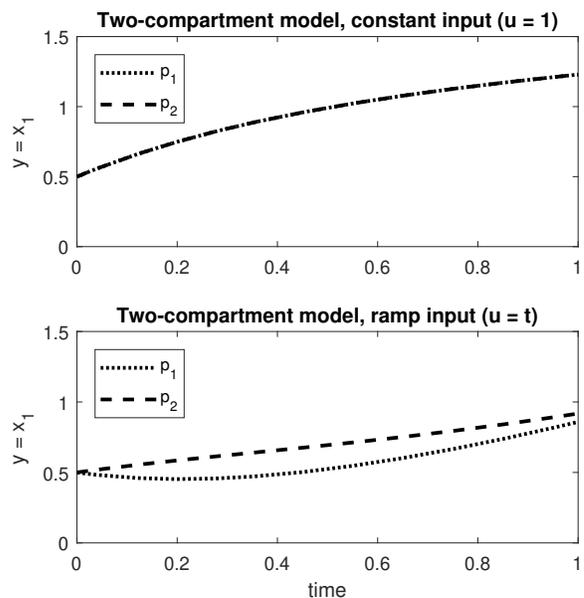


Fig. 2. Output of the two-compartment model for two different parameter vectors ( $p_1 = [1, 1, 1, 2]$ ,  $p_2 = [0.5, 0.5, 2, 1]$ ) and two different inputs (a constant input,  $u(t) = 1$ , and a ramp input,  $u(t) = t$ ). With a constant input (upper plot), the two parameter vectors yield the same model output (note that the figure plots only two parameter vectors, but there is an infinite number of pairs of such vectors that are indistinguishable) and hence the parameters are structurally unidentifiable. In contrast, the parameters become identifiable with a time-varying input such as a ramp (lower plot).

### B. A Markov model of ion channels

Ion channels regulate the flow of ions across cell membranes. Their conductance can be described by Hodgkin-Huxley (HH) models [12], and also with continuous time ODE models of Markov decision processes (MMs) [20]. Such Markov models are of the form

$$M_2 : \begin{cases} \dot{x} &= M(p, u) \cdot x, \\ y &= \sum_{i=1}^{n_1} x_i \end{cases} \quad (13)$$

where  $u$  is the membrane potential,  $x$  is the vector of  $n$  normalised conducting (open) or non-conducting (closed) states, the observation  $y$  is the sum of all of the  $n_1$  conducting states, and  $M$  is an  $n \times n$  matrix, typically sparse. The

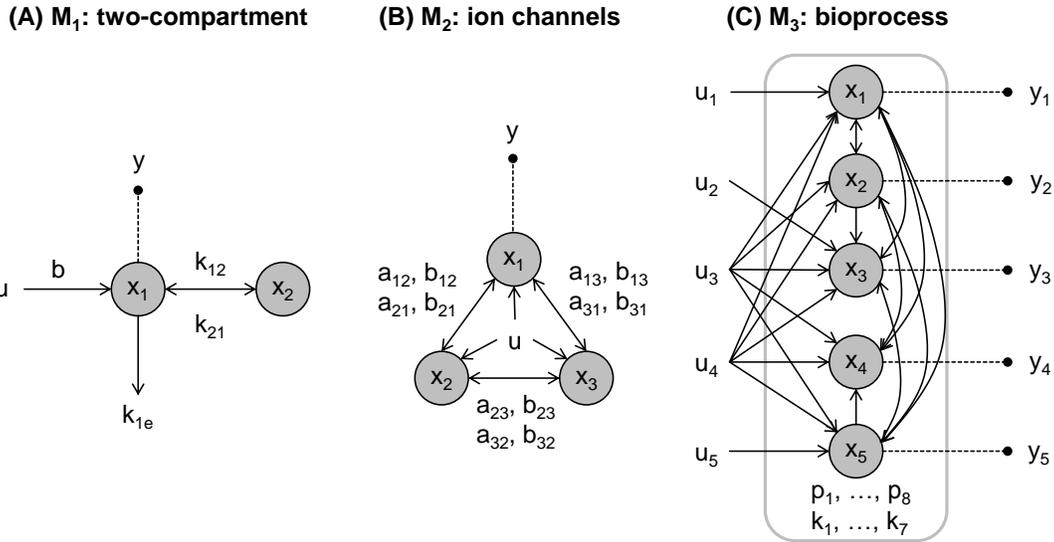


Fig. 1. Schematic representations of the three models used as case studies. Grey circles represent states ( $x_i$ ), dashed lines represent measured outputs ( $y_i$ ), and incoming arrows represent inputs ( $u_i$ ). An arrow from a state  $x_i$  to another state  $x_j$  means that  $x_i$  appears in the dynamic equation of  $\dot{x}_j$ . The remaining symbols (e.g.  $k_{12}$ ) represent unknown parameters or known constants. (A)  $M_1$ , the two-compartment model defined by (12). (B)  $M_2$ , the Markov model of ion channels defined by (13). Note that the mathematical model used in the analysis was reduced with respect to this representation, and thus it does not contain the state  $x_3$  nor parameters  $a_{31}$  and  $b_{31}$ . They are nevertheless depicted in the diagram to facilitate its interpretation. (C)  $M_3$ , the bioprocess model described in the Supplementary Information file.

matrix  $M$  is mass conserving, i.e.  $\sum_{i=1}^n x_i(t)$  is constant. In experiments the conductance  $y(t)$  changes over time as the enforced membrane potential  $u(t)$  is varied, thus altering the reaction rates. The influence of the membrane potential on the reaction rates is modelled by:

$$M_{ij}(u(t)) = \exp(a_{ij} + b_{ij} \cdot u(t)). \quad (14)$$

MMs are a generalization of HH models that are more versatile thanks to the use of more parameters. However, this increased flexibility also makes them more prone to identifiability issues: Fink and Noble analysed a number of MMs [10], concluding that most of them had some type of unidentifiability (either structural or practical).

Here we consider a cyclic MM model defined by (13,14), with  $n = 3$  states and  $y = x_1$ . In principle this model has 12 parameters (4 per state) as depicted in Fig. 1.B. However, being a cyclic model it is possible to reduce it to 2 states and 10 parameters. Its analysis reveals similar structural identifiability properties as the previous model: with a constant input it is unidentifiable, and becomes identifiable for a first order time-varying input.

For this model the requirement of a time-varying input can be replaced by 4 experiments with different constant inputs, which may be easier to perform. Indeed, past works [10] proposed experimental protocols with piecewise constant inputs for identifying this type of model. These works reported that such protocols succeeded in avoiding ‘numerical’ or ‘practical’ unidentifiability. Our result provides a theoretical justification of the success of such protocols.

### C. Bioprocess model

The nonlinear bioprocess model in Fig. 1.C has 5 states that represent gas concentrations, all of which can be measured. There are 8 unknown parameters ( $p_1, \dots, p_8$ ), and 7 known constants ( $k_1, \dots, k_7$ ), as well as 5 external inputs ( $u_1, \dots, u_5$ ). Due to lack of space, the equations of this model are provided in the Supplementary Information file, which also presents a detailed, step-by-step analysis of this model in order to illustrate the application of the methodology. In the remainder of this section we summarize the results of this analysis.

The model is structurally unidentifiable with constant inputs (specifically, the parameters  $p_4$ ,  $p_5$ ,  $p_6$ , and  $p_7$  are s.u.), while with time-varying inputs all parameters are s.l.i.. Interestingly, not *all* inputs need to be time-varying for the model to be s.l.i.; in fact, it suffices that the first derivatives of  $u_3$  and  $u_4$  are non-zero. Therefore, the model can be made structurally identifiable with constant inputs  $u_1$ ,  $u_2$ , and  $u_5$ , and ramp inputs  $u_3$  and  $u_4$ . In other words, an input vector  $u = [c_1, c_2, c_{3a} + c_{3b} \cdot t, c_{4a} + c_{4b} \cdot t, c_5]$ , where the  $c_i$  are constants, makes the model s.l.i.. Alternatively, the model can also be made s.l.i. by performing 4 experiments with different constant inputs; this is useful if experimental limitations allow only constant inputs.

## IV. CONCLUSIONS

The methodology presented in this paper analyses the structural identifiability of models with continuously time-varying inputs. To this end it uses extended Lie derivatives to include the input derivatives in the identifiability matrix. The approach takes into account the ability (or lack thereof) of a given time-varying input to excite the dynamic behaviour

in the system that leads to the resolution of structural non-identifiabilities. To the best of our knowledge, this information cannot be readily obtained with the currently available tools for structural identifiability analysis. Importantly, the methodology presented here can be applied to nonlinear and even non-rational models with multiple inputs. It should be noted that the method analyses not only structural identifiability but also nonlinear observability.

The method is helpful when designing new experiments because it delimits the type of external inputs that are required to correctly estimate the model parameters from the resulting dataset. More specifically, it establishes which derivatives of which inputs must be non-zero. It can also test the effect of a particular input. The method has been implemented as an open source MATLAB toolbox called STRIKE-GOLDD2.

We have demonstrated the methodology with three dynamic models of biological systems. The three models were classified as structurally unidentifiable from an experiment with constant input, and structurally identifiable with time-varying inputs. For two of the case studies we found that the requirement of time-varying inputs could be avoided if several experiments were performed: these models are structurally identifiable by considering four experiments with different constant inputs. This alternative can be convenient in practical applications, since it is not always possible to change the inputs to a biological system dynamically during the course of an experiment. In future work we plan to investigate this matter further, with the aim of establishing when a single experiment with time-varying inputs can be replaced by multiple experiments with constant inputs.

#### ACKNOWLEDGMENT

We thank Glenn Terje Lines (Simula) and Flavia Neddermeyer (TU Berlin) for providing models IV.B and IV.C, respectively, and Jan Hasenauer (Helmholtz Zentrum München) for helpful discussions on multi-experiment identifiability.

#### REFERENCES

- [1] M. Anguelova, J. Karlsson, and M. Jirstrand. Minimal output sets for identifiability. *Math. Biosci.*, 239(1):139–153, 2012.
- [2] E. August. Parameter identifiability and optimal experimental design. In *Int. Conf. on Computational Science and Engineering, CSE'09*, volume 1, pages 277–284, 2009.
- [3] R. Bellman and K. J. Åström. On structural identifiability. *Math. Biosci.*, 7(3):329–339, 1970.
- [4] G. Bellu, M. P. Saccomani, S. Audoly, and L. D'Angio. DAISY: a new software tool to test global identifiability of biological and physiological systems. *Comput. Methods Programs Biomed.*, 88(1):52–61, 2007.
- [5] O. Chiş, J. R. Banga, and E. Balsa-Canto. GenSSI: a software toolbox for structural identifiability analysis of biological models. *Bioinformatics*, 27(18):2610–2611, 2011.
- [6] C. Cobelli and J. DiStefano. Parameter and structural identifiability concepts and ambiguities: a critical review and analysis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 239(1):R7–R24, 1980.
- [7] L. Denis-Vidal and G. Joly-Blanchard. An easy to check criterion for (un)identifiability of uncontrolled systems and its applications. *IEEE Trans. Autom. Control*, 45:768–771, 2000.
- [8] L. Denis-Vidal, G. Joly-Blanchard, and C. Noiret. Some effective approaches to check the identifiability of uncontrolled nonlinear systems. *Math. Comput. Simul.*, 57(1):35–44, 2001.

- [9] N. D. Evans, M. J. Chapman, M. J. Chappell, and K. R. Godfrey. Identifiability of uncontrolled nonlinear rational systems. *Automatica*, 38(10):1799–1805, 2002.
- [10] M. Fink and D. Noble. Markov models for ion channels: versatility versus identifiability and speed. *Philos. Trans. Roy. Soc. London Ser. A*, 367(1896):2161–2179, 2009.
- [11] R. Hermann and A. J. Krener. Nonlinear controllability and observability. *IEEE Trans. Autom. Control*, 22(5):728–740, 1977.
- [12] A. L. Hodgkin and A. F. Huxley. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.*, 117(4):500–544, 1952.
- [13] J. Karlsson, M. Anguelova, and M. Jirstrand. An efficient method for structural identifiability analysis of large dynamic systems. In *16th IFAC Symposium on System Identification*, volume 16, pages 941–946, 2012.
- [14] T. S. Ligon, F. Fröhlich, O. T. Chiş, J. R. Banga, E. Balsa-Canto, and J. Hasenauer. Genssi 2.0: multi-experiment structural identifiability analysis of sbml models. *Bioinformatics*, page btx735, 2017.
- [15] L. Ljung and T. Glad. On global identifiability for arbitrary model parametrizations. *Automatica*, 30(2):265–276, 1994.
- [16] N. Meshkat, C. E.-z. Kuo, and J. DiStefano III. On finding and using identifiable parameter combinations in nonlinear dynamic systems biology models and combos: A novel web implementation. *PLoS One*, 9(10), 2014.
- [17] H. Pohjanpalo. System identifiability based on the power series expansion of the solution. *Math. Biosci.*, 41(1):21–33, 1978.
- [18] A. Raue, C. Kreutz, T. Maiwald, J. Bachmann, M. Schilling, U. Klingmüller, and J. Timmer. Structural and practical identifiability analysis of partially observed dynamical models by exploiting the profile likelihood. *Bioinformatics*, 25(15):1923–1929, 2009.
- [19] A. Raue, B. Steiert, M. Schelker, C. Kreutz, T. Maiwald, H. Hass, J. Vanlier, C. Tönsing, L. Adlung, R. Engesser, et al. Data2dynamics: a modeling environment tailored to parameter estimation in dynamical systems. *Bioinformatics*, 31(21):3558–3560, 2015.
- [20] Y. Rudy and J. R. Silva. Computational biology in the study of cardiac ion channels and cell electrophysiology. *Q. Rev. Biophys.*, 39(1):57–116, 2006.
- [21] M. P. Saccomani, S. Audoly, and L. D'Angio. Parameter identifiability of nonlinear systems: the role of initial conditions. *Automatica*, 39(4):619–632, 2003.
- [22] M. P. Saccomani and C. Cobelli. Qualitative experiment design in physiological system identification. *IEEE Control Syst. Mag.*, 12(6):18–23, 1992.
- [23] J. D. Stigter and J. Molenaar. A fast algorithm to assess local structural identifiability. *Automatica*, 58:118–124, 2015.
- [24] M. Vidyasagar. *Nonlinear systems analysis*. Prentice Hall, Englewood Cliffs, NJ, 1993.
- [25] A. F. Villaverde and J. R. Banga. Dynamical compensation and structural identifiability of biological models: Analysis, implications, and reconciliation. *PLoS Comput. Biol.*, 13(11):e1005878, 2017.
- [26] A. F. Villaverde and J. R. Banga. Structural properties of dynamic systems biology models: Identifiability, reachability, and initial conditions. *Processes*, 5(2):29, 2017.
- [27] A. F. Villaverde, A. Barreiro, and A. Papachristodoulou. Structural identifiability of dynamic systems biology models. *PLoS Comput. Biol.*, 12(10):e1005153, 2016.
- [28] A. F. Villaverde, N. D. Evans, M. J. Chappell, and J. R. Banga. Sufficiently exciting inputs for structurally identifiable systems biology models. *IFAC-PapersOnLine*, in press, 2018.
- [29] É. Walter and L. Pronzato. Qualitative and quantitative experiment design for phenomenological models—a survey. *Automatica*, 26(2):195–213, 1990.
- [30] E. Walter and L. Pronzato. *Identification of parametric models from experimental data*. Communications and Control Engineering Series. Springer, London, UK, 1997.
- [31] X. Xia and C. H. Moog. Identifiability of nonlinear systems with application to HIV/AIDS models. *IEEE Trans. Autom. Control*, 48(2):330–336, 2003.
- [32] J. W. Yates, N. D. Evans, and M. J. Chappell. Structural identifiability analysis via symmetries of differential equations. *Automatica*, 45(11):2585–2591, 2009.